

AA34

What is claimed is:

CLAIMS

in response to the second written opinion.

1. Use of a complex nutrient medium for the manufacture or production of a composition for topical use, the said complex nutrient medium consisting of at least some amino acids, a vitamin, a cell growth factor and an inorganic salt, and excluding any biological extract of animal origin or of cellular origin, the said medium permitting, on its own and in an aqueous medium, viable in vitro culture of an inoculum of human epidermal keratinocytes, with at least one clonal proliferation of the latter at the first passage, without a living nourishing substrate.
2. Use according to Claim 1, characterized in that the components of the nutrient medium are both biocompatible, biomimetic and bioavailable in respect of the skin.
3. Use according to Claim 1, characterized in that the complex nutrient medium has the following composition, the concentration of the components being expressed in mg/l:

**Amino acids**

L-Alanine	9.2
L-Arginine HCL [sic]	421.4
L-Asparagine (anhydrous)	14.2
25 L-Aspartic acid	4.0
L-Cysteine HCL [sic]. H <sub>2</sub> O	42.0

	L-Glutamic acid	14.8
	L-Glutamine	1754.4
	Glycine	7.6
	L-Histidine HCL [sic]. H <sub>2</sub> O	50.0
5	L-Isoleucine	6.0
	L-Leucine	131.2
	L-Lysine HCl	54.0
	L-Methionine	13.5
	L-Phenylalanine	10.0
10	L-Proline	34.6
	L-Serine	126.1
	L-Threonine	24.0
	L-Tryptophan	9.3
	L-Tyrosine 2 Na 2H <sub>2</sub> O	11.7
15	L-Valine	70.3
	<b>Vitamins and cell growth factors</b>	
	d-Biotin	0.02
	Folic acid	0.80
	Nicotinamide	0.04
20	Ca D-Pantothenate	0.30
	Pyridoxine HCl	0.06
	Riboflavin	0.04
	Thiamine HCl	0.30
	Vitamin B <sub>12</sub>	0.41
25	i-Inositol	18.0
	Putrescine 2 HCl	0.20
	Sodium pyruvate	55.0
	Thymidine	0.73
	Adenine (HCl)	24.0
30	DL-Lipoic acid	0.20

Inorganic components	
Sodium chloride	6800.0
KCl	112.0
Na <sub>2</sub> HPO <sub>4</sub>	284.0
5 CuSO <sub>4</sub> .5H <sub>2</sub> O	0.003
Sodium acetate	300.0 (anhydrous)
D-Glucose	1080.0
HEPES (piperazine)	6600.0
Phosphorylethanamine	0.06768
10 Ethanolamine	0.04684
Sodium sulphate	3.4
Sodium bicarbonate	1160.0
FeSO <sub>4</sub> .7H <sub>2</sub> O	1.39
MgCl <sub>2</sub> .6H <sub>2</sub> O	120.0
15 CaCl <sub>2</sub> .2H <sub>2</sub> O	from 13.0 to 22.05
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.144
(NH <sub>4</sub> ) <sub>6</sub> MO <sub>7</sub> O <sub>24</sub> .4H <sub>2</sub> O	0.00120
Na <sub>2</sub> SiO <sub>3</sub> .5H <sub>2</sub> O	0.142
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.00002
20 SnCl <sub>2</sub> .2H <sub>2</sub> O	0.00011
NH <sub>4</sub> VO <sub>3</sub>	0.00057

4. Composition for topical use comprising a phase which is biocompatible with the superficial parts of the human body, in which phase at least one nutrient medium as defined according to any ~~one of Claims 1 to 3~~ is distributed homogeneously.

5. Composition according to Claim 4, characterized in that it is in two-phase form, with an aqueous continuous phase containing the complex nutrient medium, and in particular in the form of an aqueous gel or an oil-in-water emulsion.

6. Composition according to Claim 4, characterized in that it is in two-phase form, with an oily continuous phase, in particular in emulsion form, the discontinuous phase containing the complex nutrient medium.

7. Cosmetic base comprising a composition according to any ~~one of Claims 4 to 6~~.

8. Cosmetic preparation comprising a cosmetic base according to Claim 7, characterized in that the complex nutrient medium constitutes either an active principle, or an excipient, in particular one that potentiates 5 another active principle.

9. Use of a complex nutrient medium having a composition, excluding any biological extract of animal origin or of cellular origin, suitable for permitting, on its own and in an aqueous medium, viable *in vitro* culture 10 of an inoculum of human epidermal keratinocytes, with at least one clonal proliferation of the latter at the first passage, without a living nourishing substrate, for the manufacture or production of a medicament.

10. Use according to Claim 9, characterized in that 15 the compounds [sic] of the nutrient medium are both biocompatible, biomimetic and bioavailable in respect of the skin.

11. Use according to Claim 9, characterized in that the complex nutrient medium has the following composition, the concentration of the components being expressed 20 in mg/l:

**Amino acids**

L-Alanine	9.2
L-Arginine HCl	421.4
25 L-Asparagine (anhydrous)	14.2
L-Aspartic acid	4.0
L-Cysteine HCl.H <sub>2</sub> O	42.0

L-Glutamic acid	14.8
L-Glutamine	1754.4
Glycine	7.6
L-Histidine HCl.H <sub>2</sub> O	50.0
5 L-Isoleucine	6.0
L-Leucine	131.2
L-Lysine HCl	54.0
L-Methionine	13.5
L-Phenylalanine	10.0
10 L-Proline	34.6
L-Serine	126.1
L-Threonine	24.0
L-Tryptophan	9.3
L-Tyrosine 2 Na 2H <sub>2</sub> O	11.7
15 L-Valine	70.3
<b>Vitamins and cell growth factors</b>	
d-Biotin	0.02
Folic acid	0.80
Nicotinamide	0.04
20 Ca D-Pantothenate	0.30
Pyridoxine HCl	0.06
Riboflavin	0.04
Thiamine HCl	0.30
Vitamin B <sub>12</sub>	0.41
25 i-Inositol	18.0
Putrescine 2 HCl	0.20
Sodium pyruvate	55.0
Thymidine	0.73
Adenine (HCl)	24.0
30 DL-Lipoic acid	0.20

Inorganic components	
Sodium chloride	6800.0
KCl	112.0
Na <sub>2</sub> HPO <sub>4</sub>	284.0
5 CuSO <sub>4</sub> .5H <sub>2</sub> O	0.003
Sodium acetate	300.0 (anhydrous)
D-Glucose	1080.0
HEPES (piperazine)	6600.0
Phosphorylethanolamine	0.06768
10 Ethanolamine	0.04684
Sodium sulphate	3.4
Sodium bicarbonate	1160.0
FeSO <sub>4</sub> .7H <sub>2</sub> O	1.39
MgCl <sub>2</sub> .6H <sub>2</sub> O	120.0
15 CaCl <sub>2</sub> .2H <sub>2</sub> O	from 13.0 to 22.05
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.144
(NH <sub>4</sub> ) <sub>6</sub> MO <sub>7</sub> O <sub>24</sub> .4H <sub>2</sub> O	0.00120
Na <sub>2</sub> SiO <sub>3</sub> .5H <sub>2</sub> O	0.142
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.00002
20 SnCl <sub>2</sub> .2H <sub>2</sub> O	0.00011
NH <sub>4</sub> VO <sub>3</sub>	0.00057

AA 12. Use according to any one of Claims 9 to 11, characterized in that the nutritional agent constitutes one of the active principles, if not the active principle, of the said medicament.

AC 13. Use according to any one of Claims 9 to 12, for obtaining a medicament intended for the preservative treatment of grafts.

AA 14. Use according to any one of Claims 9 to 13, for preventing or treating disorders and/or delay of cicatrization.

15. Medicinal composition for topical use, comprising a phase which is biocompatible with the superficial parts of the human body, in which phase at least one nutrient medium as defined according to any one of Claims 9 to 11 is distributed homogeneously.

AC 35 16. Composition according to Claim 15, characterized

in that it is in two-phase form, with an aqueous continuous phase containing the complex nutrient medium, and in particular in the form of an aqueous gel or an oil-in-water emulsion.

- 5 17. Composition according to Claim 15, characterized in that it is in two-phase form, with an oily continuous phase, in particular in emulsion form, the discontinuous phase containing the complex nutrient medium.
- 10 18. Pharmaceutical formulation base comprising a composition according to any one of ~~Claims 15 to 17~~.
- 20 [sic]. Pharmaceutical formulation base according to Claim 18, characterized in that it is intended for the preservative treatment of grafts.
- 21 [sic]. Pharmaceutical formulation base according to  
15 Claim 18, characterized in that it is intended for the prevention or treatment of disorders and/or delay of cicatrization.

Add A1

AMENDED SHEET

Add B3

Add C2